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Lyrene, Paul M., "Hybridization and introgression between deerberries and blueberries: Problems and progress" (2018). *North American Blueberry Research and Extension Workers Conference*. 9.
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Hybridization and Introgression between Deerberries and Blueberries: Problems and Progress

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Abstract:

Several hundred F₁ *Vaccinium* intersectional hybrids were produced between sections *Cyanococcus* and *Polycodium*. Tetraploid highbush blueberry cultivars (section *Cyanococcus* hybrids based on *V. corymbosum*) were the seed parents and colchicine-induced tetraploids of tall-growing (2-5m) deerberry plants (*Vaccinium stamineum*) from north-central Florida served as the pollen parents. Nine selected F₁ hybrids were backcrossed to highbush cultivars, and more than 2,000 of the resulting BC₁ seedlings were evaluated in field nurseries. Fifty-seven of the most vigorous BC₁ seedlings were selected. Some were transplanted to 20-liter pots, others to a field with drip irrigation at 1 m x 3 m spacing. All were evaluated a year later for plant and berry characteristics after open pollination. BC₁ plant architecture was similar to that of highbush, but most plants were less vigorous. Flowers on many plants had short corollas and exerted stigmas, making pollen, stigmas, and nectar more accessible to honeybees than they are in highbush cultivars. Plants flowered heavily, and fruit set was as high as is typical in commercial fields of highbush cultivars; almost every flower made a berry. Berries were fully seeded. Berries ripened 2 to 4 weeks later than highbush, were as large as highbush berries, and had green-white pulp and medium to high firmness. Berries on most BC₁ plants had black, somewhat tough skins, but berries on some selections were glaucous. Stemminess varied from near zero to 30%, with low to medium berry shattering. Picking scars ranged from small to large. Berry flavor averaged lower than in highbush. The main drawback with flavor was low sugar. Some clones had aromatic flavor components, not always pleasant. Berries on a few plants were near cultivar quality. Possible benefits from *V. stamineum* introgression include flowers with short, open, corollas (which could facilitate pollination and increase fruit set), red to purple berry pulp, (seen in some F₁ but no BC₁ plants), open flower clusters, and increased drought tolerance.

Index words: *Vaccinium stamineum*, *Vaccinium* section *Polycodium*, deerberry, blueberry breeding, intersectional hybrids, blueberry flavors.

Introduction

Vaccinium stamineum, common names “deerberry” and “gooseberry”, is a highly-variable diploid species in section *Polycodium*. It is native in eastern North America, ranging from the central Florida peninsula to extreme southeastern Canada (Ashe, 1931; Camp, 1945; Uttal, 1987; Vander Kloet 1988). The plants are deciduous, with a chilling requirement that varies from low in Florida to a high in plants from areas with cold winters. Plants range from low-growing, rhizomatous colonies to nearly monopodial, tall-growing forms that resemble highbush (*V. corymbosum* hybrids) and rabbiteye (*V. virgatum*) cultivars in plant architecture (Baker, 1970; Ballington, 1995). Both forms occur in Florida, but depending on location, one form or the other may predominate. In Florida, deerberry plants are typically found on excessively-drained soils of coarse, white sand, often in the understory of longleaf pine and xerophytic oaks. The plants re-sprout readily after fire, but at least with the tall-growing forms,

they produce few flowers or fruit if forests are burned more frequently than once per five to seven years. Because of rapid urbanization, irrigated agriculture on sandy lands, and excessive use of fire in managing public lands in the southeastern U.S., much diversity of *V. stamineum* is being lost.

Several workers have noted the potential for domestication of deerberries as a new crop (Sharpe and Sherman, 1971; Ballington et al., 1984; Ballington, 1995). Several characteristics of *V. stamineum* favor domestication. The plants produce large, juicy berries, with high soluble solids (Luby et al., 1991) and small seeds. Some plants produce berries with red flesh and bright red juice (Ballinger et al., 1981). Berry yields are frequently high, even on plants growing in the forest on drought-prone sands of low fertility. High plant-to-plant variability in traits of potential horticultural importance would favor progress in breeding.

Factors that have discouraged previous domestication of the deerberry include bitterness or astringency in the fruit of most plants, particularly in the skins, tough skins, the tendency of the berries to drop from the plant with the pedicel attached shortly after ripening, and lack of a convenient way to clone individual superior plants.

Diploid seedlings from intersectional *Vaccinium* crosses, if they can be obtained, are mostly weak and sterile (Lyrene and Ballington, 1986). At the tetraploid level, some intersectional hybrids have given some seedlings vigorous enough and fertile enough to be used in further breeding (Rousi, 1963; Lyrene, 2013; Tsuda et al., 2013)

Two factors have limited the use of deerberries in highbush blueberry breeding. First, *V. stamineum* is only distantly related to section *Cyanococcus*, which includes lowbush (*V. angustifolium*), highbush (*V. corymbosum*), rabbiteye (*V. virgatum*) and their close diploid, tetraploid, and hexaploid relatives. Second, all populations of *V. stamineum* that have been studied cytogenetically have been diploid, whereas cultivated blueberries are tetraploid or hexaploid. The diploid-tetraploid barrier to interspecific hybridization is formidable in *Vaccinium*, especially if the diploid species makes few unreduced gametes (Ballington, 1980).

The purpose of the project described here is to combine the *V. stamineum* gene pool with the cultivated highbush blueberry gene pool at the tetraploid level. The goal is to produce a fertile, tetraploid population based on highbush x *V. stamineum* hybrids, which has a broad genetic base and includes genes from many different *V. stamineum* genotypes. Such a population would make available to breeders new characteristics not easily obtained from section *Cyanococcus*, such as flowers with short, widely-open corollas, berries with red juice, berries with exotic flavors not available in *Cyanococcus*, berries that ripen late on very low-chill plants, and adaptation to low-organic soils.

Materials and Methods

The materials and methods used to initiate this project were described earlier (Lyrene, 2016; 2018). The *V. stamineum* plants used as parents came from a tall-growing (2 to 5 m) race that occurs naturally on droughty soils in the north-central Florida peninsula. The architecture of these plants is similar to that of rabbiteye blueberry. Open-pollinated seeds of plants that had high yields of large berries that remained attached to the plant after ripening and had minimum bitterness were collected from the forest. Seedlings were grown, and tetraploid plants were obtained by colchicine treatment (Lyrene, 2016). F₁ hybrids were produced in a greenhouse by pollinating emasculated flowers of many different cultivated highbush selections from the University of Florida blueberry breeding program with pollen from tetraploid *V. stamineum*

plants. The seeds were germinated on peat in a greenhouse, and the seedlings were transplanted to a high-density field nursery (Sherman et al., 1973) when 6 months old. After 18 months in the field, the strongest plants that clearly showed the phenotype of intersectional hybrids were selected, potted, and pollinated in a greenhouse with highbush pollen to produce BC₁ populations. Several thousand BC₁ plants were evaluated in high-density field nurseries. F₂ seedlings were produced by intercrossing F₁ plants, and more than 1,000 seedlings are being evaluated in field nurseries. About 1,000 BC₁ seedlings were produced in which tetraploid *V. stamineum* was the recurrent parent, and these seedlings are also being evaluated in a high-density field nursery.

Fifty-seven of the strongest BC₁ seedlings (highbush cultivars were the recurrent parent) were evaluated in their second fruiting year, some in a field at 1m x 3m spacing with drip irrigation and others in 20-liter pots placed at an outside location where most insect pollination would be BC₁ x BC₁. Berry size, berry skin color, berry flesh color, berry flavor, tendency of ripe berries to shatter, and leaf health were noted for each plant.

Results and Discussion

Eight genetically distinct tetraploid *V. stamineum* seedlings were successfully used as pollen parents in crosses with highbush cultivars. Several of the *V. stamineum* plants that had been selected as tetraploids after colchicine treatment turned out to be sectorial chimeras. Some canes (presumably tetraploid) produced large flowers with large pollen, and other canes on the same plant produced smaller flowers with normal-size pollen. Failure to recognize the chimera problem reduced the output of F₁ seedlings during the first several years of crossing. Another problem was that highbush x *V. stamineum* pollinations produced relatively few seedlings, even when the pollen came from tetraploid branches. During the first years when crosses were being made, flowers not used in crosses were allowed to remain on the highbush plants that served as female parents, under the assumption that they would not produce seeds in a bee-exclusion greenhouse. However, it became obvious in the seedling evaluation nurseries that some populations that were expected to contain only intersectional hybrids turned out to consist solely of highbush seedlings or mixtures of highbush seedlings and intersectional hybrids. Because highbush seedlings were much more vigorous than the intersectional hybrids, they had to be identified and removed from the high-density evaluation nursery during the second year or the valid intersectional hybrids would be killed by competition.

F₁ populations varied considerably in vigor, depending on the particular highbush and *V. stamineum* parents used. Almost all F₁ intersectional hybrids grew poorly in the high-density field nurseries compared to both highbush and *V. stamineum* seedlings. The leaves of most F₁ seedlings maintained an unhealthy bronze color throughout the growing season. Some parent combinations gave extremely weak seedlings. Other combinations produced a considerable number of hybrids that were strong enough to flower when 2 years old from seed. About 50 different F₁ intersectional hybrid plants produced fruit in the greenhouse after being pollinated with highbush pollen (Lyrene, 2016). The F₁ hybrids were extremely variable, especially in berry characteristics, but gave surprisingly high percent fruit set after hand pollination with highbush pollen, approaching 100% for most F₁ plants.

The number of seedlings produced per pollinated flower was compared for various types of crosses in the greenhouse. Highbush x tetraploid *V. stamineum* gave the fewest, F₁ x highbush gave more, and highbush x highbush gave most (Lyrene, 2016).

In comparing the vigor of seedling populations in the high-density nursery, the F₁ intersectional hybrids were weakest, F₂ seedlings were intermediate in vigor, and BC₁ (to highbush) seedlings were most vigorous. Both the F₂ and BC₁ populations included a few seedlings that were as vigorous as typical highbush and *V. stamineum* seedlings, but most were less vigorous.

The 57 BC₁ plants that were evaluated after open pollination had very high percent fruit set, similar to highbush cultivars. *V. stamineum* flowers have very short corollas, with both stigmas and anther tubules extending beyond the corolla tube (Cane et al, 1985). This architecture makes both nectar and pollen more accessible to honeybees than in highbush blueberry flowers. Flower structure varied widely among BC₁ plants, but flowers on most plants had shorter corollas and wider corolla apertures than the flowers of most highbush blueberry cultivars. Flower and berry clusters on most plants were unusually loose, with long pedicels and peduncles. Berry size on most plants was similar to or larger than on the smaller-fruited highbush cultivars such as 'Biloxi', 'Reveille', and 'Snowchaser'. Berries on some plants were highly glaucous, but berries on most plants were dark, but not shiny black. Shattering of ripe berries was not a problem on most seedlings. None of the plants had red berry flesh; however, it should be noted that only two of the eight *V. stamineum* plants used in the original crosses had red flesh. Berry flavor was the most disappointing aspect of most BC₁ plants. The berries of most plants were low in sugar and low in acid. However, a few plants had pleasant flavors, with good sugar/acid ratio and interesting aromas. About 25% of the plants suffered from serious leaf diseases by the time the berries were ripe in June.

The low vigor of the F₁ intersectional hybrids probably indicates that sections *Cyanococcus* and *Polycodium* are not closely related. The greatly increased vigor of F₂ populations, produced by F₁ x F₁ crosses, most likely resulted because the plants used as parents were the most vigorous F₁ plants from a large population of F₁ plants available. Furthermore, most F₁ plants had only 5 to 30% viable pollen (Lyrene, 2016), as estimated by microscopic examination, so both gametic and zygotic selection probably contributed to the higher vigor of the F₂ populations. Because large F₂ populations are much easier to produce than large F₁ populations, and because F₂ seedlings are much more vigorous than F₁ seedlings, selecting the best F₂ seedlings before backcrossing to highbush cultivars may be a more efficient breeding strategy than making backcrosses using F₁ plants.

Only a few BC₁ plants in which tetraploid *V. stamineum* was the recurrent parent have been evaluated for fruit quality. These plants were evaluated in a greenhouse after being hand-pollinated with pollen from highbush cultivars or from tetraploid *V. stamineum*. About half of these BC₁ plants set fruit well after these pollinations, and the berries contained 5 to 10 good seeds per berry. The berries, as expected, were more like deerberries than like blueberries.

Conclusions

Vaccinium stamineum is a promising species to use in breeding tetraploid, low-chill highbush blueberry cultivars for Florida because it is well-adapted to local soils and climate, has highbush architecture, and produces berries that are large and juicy with high soluble solids and small seeds, and, in some plants, red or purple flesh and juice. Based on progress so far, I have high confidence that useful new traits could be incorporated into tetraploid highbush cultivars by recurrent selection starting with broad-based highbush x *V. stamineum* hybrid populations.

Obtaining useful cultivars after crossing highbush cultivars with *V. stamineum* will not be easy. Considerable work will be involved in producing and evaluating the needed genotypes.

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Fig 1. Flowers of one F_1 hybrid between a highbush cultivar and tetraploid *V. stamineum* 4 days before anthesis and 2 days after anthesis. In nearly all F_1 hybrids, the stigma is exserted from the corolla tube before anthesis, a *V. stamineum* characteristic.



Fig. 2. Berries ripening on a plant of *V. stamineum* in the forest in northeast Florida.